



## Effects of elevated atmospheric CO<sub>2</sub>, prolonged summer drought and temperature increase on N<sub>2</sub>O and CH<sub>4</sub> fluxes in a temperate heathland

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**Title: Effects of elevated atmospheric CO<sub>2</sub>, prolonged summer drought and temperature increase on N<sub>2</sub>O and CH<sub>4</sub> fluxes in a temperate heathland**

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## Abstract

In temperate regions, climate change is predicted to increase annual mean temperature and intensify the duration and frequency of summer droughts, which together with elevated atmospheric carbon dioxide (CO<sub>2</sub>) concentrations, may affect the exchange of nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>) between terrestrial ecosystems and the atmosphere. We report results from the CLIMAITE experiment, where the effects of these three climate change parameters were investigated solely and in all combinations in a temperate heathland. Field measurements of N<sub>2</sub>O and CH<sub>4</sub> fluxes took place 1-2 years after the climate change manipulations were initiated. The soil was generally a net sink for atmospheric CH<sub>4</sub>. Elevated temperature (T) increased the CH<sub>4</sub> uptake by on average 10 µg C m<sup>-2</sup> h<sup>-1</sup>, corresponding to a rise in the uptake rate of about 20 %. However, during winter elevated CO<sub>2</sub> (CO<sub>2</sub>) reduced the CH<sub>4</sub> uptake, which outweighed the positive effect of warming when analyzed across the study period. Emissions of N<sub>2</sub>O were generally low (<10 µg N m<sup>-2</sup> h<sup>-1</sup>). As single experimental factors, elevated CO<sub>2</sub>, temperature and summer drought (D) had no major effect on the N<sub>2</sub>O fluxes, but the combination of CO<sub>2</sub> and warming (TCO<sub>2</sub>) stimulated N<sub>2</sub>O emission, whereas the N<sub>2</sub>O emission ceased when CO<sub>2</sub> was combined with drought (DCO<sub>2</sub>). We suggest that these N<sub>2</sub>O responses are related to increased rhizodeposition under elevated CO<sub>2</sub> combined with increased and reduced nitrogen turnover rates caused by warming and drought, respectively. The N<sub>2</sub>O flux in the multifactor treatment TDCO<sub>2</sub> was not different from the ambient control treatment. Overall, our study suggests that in the future, CH<sub>4</sub> uptake may increase slightly, while N<sub>2</sub>O emission will remain unchanged in temperate ecosystems on well-aerated soils. However, we propose that continued exposure to altered climate could potentially change the greenhouse gas flux pattern in the investigated heathland.

## 1. Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) and methane ( $\text{CH}_4$ ) are important greenhouse gases (IPCC, 2007), and  $\text{N}_2\text{O}$  is the dominant substance responsible for depletion of the stratospheric ozone layer (Ravishankara et al., 2009). On a global scale,  $\text{N}_2\text{O}$  emissions from unfertilized grassland/steppe, including heathlands, is estimated to be  $0.4 \text{ Tg N}_2\text{O-N y}^{-1}$  (Stehfest and Bouwman, 2006), which corresponds to about 2% of the total annual  $\text{N}_2\text{O}$  emissions (Fowler et al., 2009). Methane uptake by aerobic soils worldwide is estimated to be  $30 \text{ Tg CH}_4 \text{ y}^{-1}$ , counteracting 6% of the total emission of  $\text{CH}_4$  from natural and anthropogenic sources (Wuebbles and Hayhoe, 2002).

Nitrous oxide emitted from soil primarily originates from the two microbial processes nitrification and denitrification, which occur under aerobic and anaerobic soil conditions, respectively (Wrage et al., 2001). Thus, both processes are controlled by soil moisture that regulates oxygen ( $\text{O}_2$ ) availability and by supply of substrates, ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ). In addition, denitrifying bacteria need labile carbon (C) compounds as an energy source. Soils may act as a sink for atmospheric  $\text{N}_2\text{O}$ , which has been observed at low mineral nitrogen (N) availability and the responsible organisms could be denitrifying bacteria, but are probably also nitrifying organisms (Chapuis-Lardy et al., 2007).

The  $\text{CH}_4$  flux between soil and atmosphere is the net result of  $\text{CH}_4$  production by methanogenic archaea and  $\text{CH}_4$  oxidation by methanotrophic bacteria (Le Mer and Roger, 2001). In aerobic soils,  $\text{CH}_4$  oxidation typically proceeds at a greater rate than  $\text{CH}_4$  production, resulting in net uptake of atmospheric  $\text{CH}_4$ . Maximum  $\text{CH}_4$  oxidation usually occurs in deeper soil layers. Thus,  $\text{CH}_4$  uptake is strongly controlled by the physical diffusion of atmospheric  $\text{CH}_4$  through the soil profile, which is mainly regulated by the soil texture and water content (King, 1997), as molecular diffusion in water is four orders of magnitude slower than in air.

The effect of drought on fluxes of CH<sub>4</sub> and N<sub>2</sub>O has only been investigated in a limited number of studies in temperate ecosystems on aerobic soils. In a temperate spruce forest, Borken et al. (2000) found that prolonged summer drought increased the annual CH<sub>4</sub> uptake by more than 40%. However, in a deciduous forest on well-drained soil, the CH<sub>4</sub> uptake was only increased by 7% because soil in the control plots already had a low water content due to effective drainage (Borken et al., 2006). Goldberg and Gebauer (2009) reported that summer drought reduced the N<sub>2</sub>O emission from a spruce forest.

Elevated atmospheric carbon dioxide (CO<sub>2</sub>) concentrations affect soil properties via plant-mediated processes, and thereby potentially the fluxes of N<sub>2</sub>O and CH<sub>4</sub>. It is well known that plants growing under elevated CO<sub>2</sub> may reduce their transpiration, leading to increased water use efficiency and soil moisture contents (Morgan et al., 2004; Robredo et al., 2007). Furthermore, a common plant response to elevated CO<sub>2</sub> is increased deposition of root-derived C into the rhizosphere (Allard et al., 2006; Pendall et al., 2004).

The effect of elevated atmospheric CO<sub>2</sub> on N<sub>2</sub>O fluxes has been investigated intensively through the last decade in a variety of ecosystems. Long-term studies ( $\geq 1$  year) in natural or semi-natural ecosystems on well-aerated soils with low N availability show either no response in annual N<sub>2</sub>O emissions rates to atmospheric CO<sub>2</sub> levels (Baggs et al., 2003; Mosier et al., 2002; Phillips et al., 2001b) or increased N<sub>2</sub>O emissions from soil under elevated CO<sub>2</sub> (Kammann et al., 2008). The positive effect of elevated CO<sub>2</sub> on the N<sub>2</sub>O emission was explained by increased rhizodeposition of labile C substrates stimulating denitrification. In an N-limited pine forest, Phillips et al. (2001b) observed seasonal variability in the N<sub>2</sub>O response to elevated atmospheric CO<sub>2</sub> compared to an ambient control. This included reduced N<sub>2</sub>O emissions during the growing season due to high plant-microbial competition for N, and enhanced N<sub>2</sub>O emissions during winter possibly because denitrification was stimulated by greater soil moisture and labile C sources

under elevated CO<sub>2</sub>. In a short-term study during autumn, Arnone and Bohlen (1998) also found that elevated atmospheric CO<sub>2</sub> increased N<sub>2</sub>O emission, which they ascribed to improved soil moisture conditions in a relatively dry grassland favouring the microbial transformation of N.

Reduced CH<sub>4</sub> consumption under elevated atmospheric CO<sub>2</sub> has been observed in several ecosystems on undisturbed aerobic soils (Ambus and Robertson, 1999; Baggs and Blum, 2004; Dubbs and Whalen, 2010; Ineson et al., 1998; Phillips et al., 2001a), but the exact mechanism is not well known and may vary between ecosystems. Two possible mechanisms have been suggested for the reducing effect of elevated CO<sub>2</sub> on net CH<sub>4</sub> consumption: i) decreased CH<sub>4</sub> oxidation due to higher soil water content and thereby reduced diffusion of CH<sub>4</sub> (Ambus and Robertson, 1999; Baggs and Blum, 2004) and ii) increased CH<sub>4</sub> production due to anaerobic microsites caused by reduced O<sub>2</sub> diffusion and increased respiration (McLain and Ahmann, 2008).

Effects of elevated temperature on N<sub>2</sub>O and CH<sub>4</sub> fluxes have only been investigated in a few field-scale warming experiments located in temperate ecosystems on well-aerated soils (McHale et al., 1998; Peterjohn et al., 1994; Rustad and Fernandez, 1998). The artificial warming had either no effect on CH<sub>4</sub> flux or it increased CH<sub>4</sub> uptake, which could be related to the observed warming-induced declines in soil moisture in certain soil layers. Warming may reduce soil moisture by increasing the evapotranspiration (Dermody et al., 2007). No effect of warming on N<sub>2</sub>O flux was observed (McHale et al., 1998; Peterjohn et al., 1994), although elevated temperature is known to increase net N mineralization (Rustad et al., 2001).

In the current study, the effects of future climatic and atmospheric conditions on the biosphere-atmosphere exchange of N<sub>2</sub>O and CH<sub>4</sub> were investigated in a temperate heathland at the CLIMAITE experimental site ([www.climaite.dk](http://www.climaite.dk)). CLIMAITE was initiated in 2005 to improve our understanding of how biological processes in natural terrestrial ecosystems may be

affected under future environmental conditions involving elevated temperature, elevated concentration of atmospheric CO<sub>2</sub> and prolonged summer drought, simulating *in situ* the climatic scenario as predicted for Denmark in year 2075 (Mikkelsen et al., 2008). Previous studies have examined the effects on greenhouse gas fluxes of warming, elevated CO<sub>2</sub> and summer drought, but to our knowledge field studies combining all three factors in a full-factorial design have not been reported. We formulated four hypotheses for the responses in N<sub>2</sub>O and CH<sub>4</sub> fluxes to the climate change parameters investigated in the experiment.

- 1) Prolonged summer drought will stimulate CH<sub>4</sub> uptake and reduce N<sub>2</sub>O emissions.
- 2) Elevated CO<sub>2</sub> will reduce CH<sub>4</sub> uptake due to higher soil moisture caused by plant water saving mechanisms. Nitrous oxide emissions will remain unchanged under elevated CO<sub>2</sub> because the stimulating effects of increased soil moisture and availability of labile carbon compounds will be offset by increased plant-microbial competition for N.
- 3) Warming will increase CH<sub>4</sub> uptake because of reduced soil moisture. On an annual basis N<sub>2</sub>O emission will be unaffected by warming, but this may include a reduction during spring and summer due to reduced soil moisture and an increased emission in autumn due to higher N turnover rates.
- 4) In the combinations of two or three treatments, the treatment effects will either counteract each other, if in opposite directions, or intensify each other, if in the same direction.

To address these hypotheses, we conducted a full-factorial study including all treatments. In addition, a more intense study involving a subset of five treatments was carried out to focus on treatment effects during the experimental summer drought and the subsequent rewetting period.

## 2. Materials and methods

### 2.1 Field site

The study took place at the CLIMAITE experimental site (Mikkelsen et al., 2008) situated at Brandbjerg (55°53' N, 11°58' E) about 50 km NW of Copenhagen, Denmark. The site is a dry, temperate heathland on a hilly nutrient-poor sandy podzol (FAO classification). The mineral soil consists of 20.5% coarse sand, 71.6% fine sand, 5.8% silt and 2.2% clay, and has a pH<sub>H2O</sub> of 4-5. Mean pore volume and field capacity of the upper 15 cm of the mineral soil is 42 and 17 vol%, respectively. The content of carbon and nitrogen decline sharply from 6.4% and 0.34% in the upper 2 cm of the mineral soil to 0.39% and 0.02% in the 10-30 cm layer, respectively. Above the mineral soil is an organic top layer of about 5 cm in depth containing approximately 23% carbon and 1.2% nitrogen. The vegetation is dominated by *Calluna vulgaris* (L.), *Deschampsia flexuosa* (L.) and various mosses. Annual mean temperature is 8.0 °C, annual mean precipitation is 613 mm (www.DMI.dk) and the N deposition is 1.25 g N m<sup>-2</sup> year<sup>-1</sup> (Ellermann et al., 2005). The study site was chosen to represent a semi-natural ecosystem having the required low vegetation to allow for experimental manipulations.

### 2.2 Experimental design

The climate change manipulations were initiated in October 2005 and consisted of eight treatments, viz. elevated temperature (T), prolonged summer drought (D), elevated atmospheric CO<sub>2</sub> concentrations (CO<sub>2</sub>), all combinations of these treatments (TD, TCO<sub>2</sub>, DCO<sub>2</sub> and TDCO<sub>2</sub>) and untreated controls (A) (Mikkelsen et al., 2008). All treatments were applied to six replicate plots, i.e. 48 plots in total. The field site covered an area of about 2 ha and the experimental plots were distributed in twelve 6.8-m diameter octagons arranged pair-wise in six blocks, where one octagon in each block was exposed to elevated CO<sub>2</sub> concentrations (Fig. 1a). Each octagon



consisted of four plots in a split design with the treatments drought or elevated temperature solely or in combination, and a non-warmed, non-drought plot (Fig. 1b). Temperature was increased by passive night-time warming using automatic horizontal curtains that withdrew in case of precipitation during night. Periods of drought were achieved using automatic curtains, which extended above the plots during rain events. The atmospheric CO<sub>2</sub> concentration was increased from the ambient level of 380 ppm to 510 ppm by the Free Air Carbon Enrichment (FACE) technique that involved feedback control by monitored CO<sub>2</sub> concentration, wind speed and wind direction. The temperature increase produced by the passive night-time warming showed a seasonal pattern. More specifically, the mean and maximum temperature increase at 5 cm soil depth was higher in spring (0.6, 1.5 °C), summer (0.5, 1.3 °C) and autumn (0.3, 1.0 °C) than in the winter (0.2, 0.7 °C). The experimental drought periods took place in July 2006 and May-June 2007, and lasted one month after which the soil water content typically reached 5 vol% in the top 20 cm of the soil.

<Figure 1>

### *2.3 N<sub>2</sub>O and CH<sub>4</sub> flux measurements*

In October 2004, one stainless steel collar (60 cm × 60 cm × 10 cm deep) for gas flux measurements was installed in each plot to a maximum depth of 7 cm. In some plots, a section of the collar could not be inserted into the soil due to shallow *Calluna* roots, and instead a substantial border of soil was placed on the outside of the collar to ensure a complete seal against bulk air flow.

The field measurements of N<sub>2</sub>O and CH<sub>4</sub> fluxes were conducted from June 2006 to October 2007 at weekly to monthly intervals. The aim was to examine treatment effects, and as

fluxes of N<sub>2</sub>O are known to be low in dry soils, the measuring effort was targeted at rain events when possible. Two studies were carried out: i) A full-factorial study including all eight treatments and ii) a drought/rewetting study in relation to the summer drought in 2007 including the treatments A, CO<sub>2</sub>, D, DCO<sub>2</sub> and TDCO<sub>2</sub>. The full-factorial study consisted of 11 measuring campaigns evenly distributed between the warm and the cold season. Each measuring campaign in the full-factorial study was conducted in four sub-campaigns over two consecutive days. Each sub-campaign included at least one replicate of each treatment in order to minimize the influence on possible treatment effects of any day-to-day fluctuation in emission rates, as well as fluctuations between morning and midday. The drought/rewetting study began in April 2007 and included only five of the treatments in order to enable the measurement of all plots on a single day. This was performed at one to 20 days intervals over a five months period (n=11).

In a measuring campaign, fluxes of N<sub>2</sub>O and CH<sub>4</sub> were determined by a static chamber method using white PVC chambers placed on the metal collars installed in the plots. During wintertime, 15-cm high chambers were used for 2-hour enclosure periods, whereas 45-cm high chambers were used during summertime for 2.5-hour enclosure periods in order not to disturb the vegetation. Each chamber had a sponge rubber edge trim, which fitted into a channel on the upper edge of the soil collar and ensured a tight seal during measurements. The chambers were equipped with a gas sampling port and a fan (50 × 50 mm) to mix the headspace air. Four times during the enclosure period, two 30-ml samples of headspace air was taken with a syringe through the sampling port and used to flush a 3.5-ml Venoject vial and a 2-ml crimp-seal vial, respectively. To measure N<sub>2</sub>O concentrations, the 3.5-ml vials were pressurized to 1.4 bar with carrier gas immediately before analysis by gas chromatography (GC-14B, Shimadzu, Kyoto, JP). The 2-ml samples for CH<sub>4</sub> determination were supplemented with 0.5 ml N<sub>2</sub> and the

concentrations were established by gas chromatography (HP 6890, Agilent, Santa Clara, US).

Nitrous oxide fluxes and CH<sub>4</sub> emissions into the chamber were calculated using linear regression of headspace concentrations versus time (n=4), whereas CH<sub>4</sub> uptake was calculated by fitting a first-order function.

#### *2.4 Soil moisture, temperature and nitrate*

Each of the 48 plots in the climate change experiment had a soil temperature probe installed at 5 cm depth recording at 1 Hz and data was logged as hourly means. Time Domain Reflectometry (TDR) probes were installed at 0-20 cm and 0-60 cm depths to measure volumetric soil water content on a half-hourly basis. More specifically, the TDR probes measure the ability of the soil to transmit an electric field, which is converted into volumetric soil moisture using a soil type specific algorithm. The soil water content in the 20-60 cm layer was calculated from the measurements in 0-20 cm and 0-60 cm. In addition, manual measurements of soil moisture at 0-6 cm depth were conducted at each soil collar during some flux measuring campaigns (ThetaMeter HH1, Delta-T Devices, Cambridge, UK). During each measurement sub-campaign, air temperature was recorded outside two gas flux chambers to be used in the flux calculations. Precipitation was recorded at 2 m height by two independent weather stations at the field site (Fig. 1a).

Soil water was collected monthly from passive PVC soil water draining collectors below the organic soil layer (approximately 5 cm depth) in each experimental plot. Concentrations of NO<sub>3</sub><sup>-</sup> were analyzed on an Autoanalyzer 3 (Bran+Luebbe GmbH, Germany).

## 2.5 Potential nitrification and denitrification

In order to evaluate the long-term effect of the summer drought treatment, soil samples were collected in late autumn, viz. on 5 November 2007. In each plot, three subsamples from the 0-8 cm soil layer under *Deschampsia flexuosa* were obtained using a 2.5-cm diameter soil core. The subsamples were pooled, gently homogenized by hand and major roots were removed. Soil samples were stored at 4 °C until analysis (less than 24 h). Potential nitrification was determined by adding 3 g fresh soil to a 100-ml Erlenmeyer bottle together with 20 ml of Winogradsky solution containing 7.6 mM ammonium sulphate. The bottle was placed on a shaker (100 rpm) and incubated at 25 °C. After 0 and 168 h, a 10-ml sample was centrifuged (3500×G; 25 °C; 5 min) and the supernatant was analyzed for nitrite and nitrate (Fiastar 5000 Flow Analyzer, FOSS, Hillerød, DK). Potential nitrification rates were estimated from the increase in nitrite+nitrate concentrations between 0 and 168 h.

To measure potential denitrification, 10 g fresh soil was placed in a 100-ml incubation bottle and 15 ml of 1 mM potassium nitrate, 0.5 mM glucose, 0.5 mM sodium acetate and 0.5 mM sodium succinate was added. The bottle was sealed with a butyl rubber stopper, flushed with N<sub>2</sub> for 2 min, had 10% acetylene added, and was then placed horizontally on a shaker (200 rpm) and incubated at 22 °C. After 30, 70, 120 and 180 min, 3 ml headspace was transferred to a pre-evacuated 3-ml Venoject vial. The vials were pressurized with 1 ml N<sub>2</sub> before analysis for N<sub>2</sub>O on a gas chromatograph (HP 5890, Agilent, Santa Clara, US). Potential denitrification rates were estimated from linear regression of total N<sub>2</sub>O, i.e. headspace and aqueous phase N<sub>2</sub>O (Weiss and Price, 1980), versus time.

## 2.6 Nitrous oxide reductase activity

To determine N<sub>2</sub>O reductase activity, NO<sub>3</sub><sup>-</sup> was removed from soil samples by vortexing 10 g fresh soil with 30 ml of phosphate buffered saline (PBS) for 5 sec of followed by centrifugation for 10 min at 3500×*G* and 5 °C. The supernatant was discarded and the pellet resuspended in 30 ml PBS. This process was repeated three times; after the last, the pellet was resuspended in 15 ml of 0.5 mM glucose, 0.5 mM sodium acetate, and 0.5 mM sodium succinate and transferred to a 100-ml incubation bottle. The bottle was sealed with a butyl rubber stopper, flushed with N<sub>2</sub> for 2 min, had 100 ppm N<sub>2</sub>O added (final concentration), and was then placed horizontally on a shaker (200 rpm) and incubated at 22 °C. After 0, 1, 3, 6 and 24 h, 3 ml of headspace was transferred to a pre-evacuated 3-ml Venoject vial before analysis of N<sub>2</sub>O as described above. The decline in headspace and aqueous phase N<sub>2</sub>O (Weiss and Price, 1980) during the incubation was used to calculate N<sub>2</sub>O reductase activity.

## 2.7 Statistics

Analyses of variance (ANOVA) were conducted by fitting mixed effects models using the PROC MIXED procedure of SAS (SAS Institute, 2003). The random effects accounted for the experimental design (*Random Block Octagon*). Data was transformed as required to obtain normality and homogeneity of variance. In the full-factorial study, all CH<sub>4</sub> analyses were performed on square root transformed fluxes, viz.  $(-\text{CH}_4 \text{ flux} + 110)^{0.5}$ , where CH<sub>4</sub> uptake rates were entered as negative values. For repeated measures, the optimal covariance structure was selected using Akaike's An Information Criterion (AIC). In order to optimise the model, main factor effects (CO<sub>2</sub>, D, T) and interactions (CO<sub>2</sub>×D, CO<sub>2</sub>×T, D×T, CO<sub>2</sub>×D×T) with  $P \leq 0.25$  were

kept in the model during the step-wise reduction of the fixed effects in the model. Effects with  $P \leq 0.05$  were considered to be significant.

Data from the full-factorial study and the drought/rewetting study were analyzed separately. The statistical analysis of the full-factorial study consisted of two parts. First, treatment effects on  $\text{CH}_4$  and  $\text{N}_2\text{O}$  fluxes were examined with repeated measures models that also tested for treatment effects at specific measuring campaigns ( $\text{CO}_2 \times \text{D} \times \text{T} \times \text{Time}$ ). Differences of least squares means was used to interpret significant treatment interactions and estimate absolute changes in response to the main treatments. Soil moisture and mean daytime soil temperature (sunrise to sunset) recorded on dates of the full-factorial study campaigns were analyzed in the same way. Secondly, other repeated measures models were used to evaluate how  $\text{N}_2\text{O}$  and  $\text{CH}_4$  fluxes were influenced by the two covariates, soil moisture and daytime soil temperature. Dependency between soil moisture and soil temperature was determined by Pearson's Correlation Coefficient using the PROC CORR procedure of SAS.

Data from the drought/rewetting study was analyzed sequentially examining one measuring campaign at the time using a two-factor model including D,  $\text{CO}_2$  and  $\text{CO}_2 \times \text{D}$  (dataset excluding  $\text{TDCO}_2$ ). In addition, single factor repeated measures models were used to analyze the  $\text{N}_2\text{O}$  and  $\text{CH}_4$  fluxes across the drought and rewetting periods with time or soil moisture as covariates (dataset including  $\text{TDCO}_2$ ).

### **3 Results**

#### *3.1 Soil moisture and temperature in the full-factorial study*

Compared to the annual mean temperature and precipitation for the site, the years 2006 and 2007 where the study took place were generally warmer and wetter with a mean temperature of  $10.2^\circ\text{C}$  and an annual precipitation of 746 mm. Rain events were distributed throughout the

experimental period, but heavy rainfall was absent in the spring of 2007 (Fig. 2a). The soil water content was generally higher in the 0-20 cm soil layer than in the 20-60 cm layer (Fig. 2b). Mean daytime soil temperature at 5 cm depth measured during the full-factorial study campaigns showed a steady decline from July 2006 to March 2007 (Fig. 2c). The passive night-time warming increased the mean daytime soil temperature by 0.39 °C on average ( $P<0.0001$ ) and the temperature was slightly lower in drought treated plots compared to non-drought treated plots ( $P=0.05$ ; Table 1). Furthermore, the CO<sub>2</sub> treatment increased soil temperature but only in unwarmed plots (CO<sub>2</sub>×T;  $P<0.0001$ ). This CO<sub>2</sub> effect could be due to reduced water consumption by plants in response to elevated atmospheric CO<sub>2</sub> and thereby reduced loss of heat. Soil moisture in the upper 20 cm was affected by the drought treatment at three measuring campaigns during and following the experimental droughts, resulting in a 0.85 vol% lower soil water content in the drought treated compared to the non-drought treated plots on average across the study period ( $P=0.0004$ ; Table 1). The drought effect on soil moisture was most pronounced in plots at ambient CO<sub>2</sub> (CO<sub>2</sub>×D;  $P=0.0017$ ). Warming also had an effect on the soil water content in the top soil during seven measuring campaigns, reducing it by 0.99 vol% on average ( $P<0.0001$ ). In the lower soil layer from 20 to 60 cm, drought and warming reduced the soil water content by 0.79 and 0.84 vol%, respectively ( $P\leq 0.0036$ ). In March 2007, the water content in 0-6 cm was higher in the plots exposed to elevated CO<sub>2</sub> compared to the ambient CO<sub>2</sub> plots ( $P=0.0069$ ), however soil moisture in 0-20 cm and 20-60 cm were not affected by the CO<sub>2</sub> treatment (data not shown).

<Figure 2>

<Table 1>

### 3.2 CH<sub>4</sub> fluxes in the full-factorial study

Fluxes of CH<sub>4</sub> were generally into the soil with 80% of the measured fluxes falling in the range -1 to -130  $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ . The CH<sub>4</sub> uptake was on average 10  $\mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$  higher in the warmed plots compared to the unwarmed plots ( $P=0.044$ ) (Fig. 3a, Fig. 4a). Furthermore, an interaction appeared between the CO<sub>2</sub> treatment and time, which was the product of the lower CH<sub>4</sub> uptake in elevated CO<sub>2</sub> plots compared to ambient CO<sub>2</sub> plots during one measuring campaign in January 2007 (CO<sub>2</sub>×Time;  $P<0.0001$ ).

We tested the effect on the CH<sub>4</sub> flux of soil moisture recorded at the start of flux measurement as well as mean daytime soil temperature. Daytime soil temperature at 5 cm depth explained the variability in CH<sub>4</sub> fluxes to a greater extent ( $P<0.0001$ ) than soil moisture in the upper 20 cm ( $P=0.0049$ ), which again explained the flux better than moisture in the 20-60 cm soil layer ( $P=0.021$ ). The less significant effect of soil moisture in the top layer compared to soil temperature could arise from the linear modelling of the relationship between soil moisture and the square root transformed CH<sub>4</sub> uptake. However, linear modelling might be misleading since several studies have indicated that the relationship is hump-shaped, *i.e.* at high soil moisture the relationship is negative because an increase in soil moisture reduces gas diffusivity, while at low soil moisture the relationship may be positive because soil moisture limits the methanotrophic activity (*e.g.* Del Grosso et al., 2000). As a test of the potential linearity, we excluded two measuring campaigns where the lowest soil moisture values were recorded, *viz.* below 6 vol%. However, excluding these data from the analysis removed the negative relationship between CH<sub>4</sub> uptake and soil moisture ( $P=0.61$ ), whereas the positive relationship with soil temperature remained ( $P=0.0083$ ). Thus, linear modelling of soil moisture and CH<sub>4</sub> uptake seems reasonable in the full-factorial study. Effects of soil temperature could be indirect via the effect of



temperature on soil moisture as the two parameters were 54% negatively correlated. However, an analysis including both soil moisture in the top layer and soil temperature supported the stronger positive effect of soil temperature ( $P=0.0004$ ) compared to the negative effect of soil moisture ( $P=0.074$ ) on the  $\text{CH}_4$  uptake (Table 2).

As stated above warming increased the  $\text{CH}_4$  uptake by  $10 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$  on average. To evaluate how much of this could be explained by changes in soil temperature and soil moisture, respectively (Table 1), the model in Table 2 was used to calculate the increase in  $\text{CH}_4$  uptake resulting from an  $0.39^\circ\text{C}$ -increase in mean soil temperature across the study period and a  $0.99 \text{ vol}\%$ -decline in mean soil water content. Based on the model, changes in soil temperature and soil moisture due to the experimental warming caused an increase in the  $\text{CH}_4$  uptake of  $0.7$  and  $0.9 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ , respectively, all together explaining 16% of the measured increase in the  $\text{CH}_4$  uptake.

<Figure 3>

<Figure 4>

<Table 2>

### 3.3 $\text{N}_2\text{O}$ fluxes in the full-factorial study

Generally, emission rates of  $\text{N}_2\text{O}$  were small ( $<10 \mu\text{g N m}^{-2} \text{ h}^{-1}$ ; Fig. 3b). The repeated measures model fitted to the  $\text{N}_2\text{O}$  fluxes revealed an interaction between the  $\text{CO}_2$  and drought treatments ( $\text{CO}_2 \times \text{D}$ ;  $P=0.047$ ). Inspection of differences of least squares means showed that this interaction arose because the  $\text{N}_2\text{O}$  emission decreased in response to drought, but only in plots that were also exposed to elevated  $\text{CO}_2$ , viz.  $\text{CO}_2 + \text{TCO}_2 > \text{DCO}_2 + \text{TDCO}_2$  (Fig. 4b). As single experimental factors elevated  $\text{CO}_2$ , drought and warming had no significant effect on the  $\text{N}_2\text{O}$

flux, but the combination of CO<sub>2</sub> and warming caused increased N<sub>2</sub>O emission in the TCO<sub>2</sub> treatment, whereas the N<sub>2</sub>O production ceased in the DCO<sub>2</sub> treatment, where CO<sub>2</sub> was combined with drought. The statistical analysis also revealed an interaction between the drought treatment and time (D×Time; P<0.0001), which was caused by reduced N<sub>2</sub>O emission from drought treated plots compared to non-drought treated plots during one measuring campaign following a rain event during the summer drought treatment in 2006. Daytime soil temperature at 5 cm depth, soil moisture in the upper 20 cm and in the 20-60 cm layer all explained the N<sub>2</sub>O flux equally well (P<0.0001).

#### 3.4 Nitrate, potential nitrification and denitrification

Mean annual NO<sub>3</sub><sup>-</sup> concentration in soil water was reduced in plots exposed to either elevated CO<sub>2</sub> or drought as single factor (CO<sub>2</sub>×D×T; P=0.0064), whereas warming as main factor increased the NO<sub>3</sub><sup>-</sup> concentration (P=0.003; Fig. 5). Soil samples collected under *Deschampsia* vegetation in November 2007 were incubated at uniform temperature conditions to assess potential nitrification and denitrification, which indicated the amount of nitrifying and denitrifying enzymes present in the 0-8 cm soil layer at the site. Potential nitrification was higher in the warmed plots than in the unwarmed plots (P=0.0003), especially in the TD treatment, whereas the activity tended to be low in plots exposed to CO<sub>2</sub> as a single factor (Fig. 6a). Potential denitrification was also increased in the warmed plots (P=0.014; Fig. 6b). Negligible N<sub>2</sub>O reductase activity was observed in the soil samples with N<sub>2</sub>O uptake rates less than 1 ng N g<sup>-1</sup> dw h<sup>-1</sup> in all samples (data not shown).

<Figure 5>

<Figure 6>

### 3.5 Soil moisture and precipitation in the drought/rewetting study

During the drought period from 21 May to 22 June 2007, 94 mm of precipitation was excluded from the drought treated plots (Fig. 7a). As a result, soil moisture in the 0-6 cm layer was lower in the drought treated compared to the non-drought treated plots during the drought period (Fig. 7b). The drought effect on soil water persisted through the subsequent rewetting period ( $P < 0.009$ ), where all plots received 53 mm of rain. Soil moisture at 0-6 cm depth peaked on the second day after rewetting, which occurred on 27 June where 25 mm of rainfall was recorded.

<Figure 7>

### 3.6 $CH_4$ and $N_2O$ fluxes in the drought/rewetting study

During the drought and rewetting periods, drought effects on the  $CH_4$  and  $N_2O$  fluxes occurred on 30 May, the first day of measurement during the drought, where both  $CH_4$  uptake and  $N_2O$  emission decreased in the drought treated compared to the non-drought treated plots ( $P \leq 0.022$ ; Fig. 7c,d). However, drought effects were absent during the last part of the drought period and during the rewetting (*i.e.* 14 June to 2 July). Repeated measures models were used to evaluate which other factors controlled the fluxes during this time span and revealed that the  $N_2O$  flux was highly controlled by the soil water content ( $P = 0.024$ ). Furthermore, the  $N_2O$  emission on the second day after rewetting was higher than the other days ( $P = 0.005$ ), thus the response in the  $N_2O$  flux to rewetting was delayed by one day. In contrast, the  $CH_4$  flux responded immediately to rewetting by a general increase in  $CH_4$  uptake across all treatments the first day

after rewetting compared to the other days ( $P=0.0087$ ). As the soil water content continued to rise, the  $\text{CH}_4$  oxidation was hindered, resulting in reduced  $\text{CH}_4$  uptake and even net  $\text{CH}_4$  emission in some plots during the last two campaigns in the rewetting period. Across the five treatments investigated, six of the 30 plots were  $\text{CH}_4$  hotspots with emission rates above  $100 \mu\text{g C m}^{-2} \text{ h}^{-1}$ . No delayed effect of the drought treatment appeared in  $\text{N}_2\text{O}$  and  $\text{CH}_4$  fluxes during four measuring campaigns from *medio* August to *primo* October 2007 (data not shown). Following 13 mm of rainfall in the beginning of October, consistently net  $\text{CH}_4$  emission was observed again with 76% of the measured flux rates being  $\text{CH}_4$  efflux.

## 4 Discussion

### 4.1 Effects of warming and elevated $\text{CO}_2$ on $\text{CH}_4$ fluxes

Methane fluxes ranged from net uptake to net emission, demonstrating that both methanotrophs ( $\text{CH}_4$ -oxidizing bacteria) and methanogens ( $\text{CH}_4$ -producing archaea) were present in the soil microbial community at the site. Generally,  $\text{CH}_4$  oxidation exceeded  $\text{CH}_4$  production with 80% of the measured flux rates in the full-factorial study falling in the range -1 to  $-130 \mu\text{g C m}^{-2} \text{ h}^{-1}$ . This is comparable to the only other study that examined  $\text{CH}_4$  fluxes in a shrubland on sandy soils, which happened to be in a Mediterranean sclerophyllous shrubland, where the  $\text{CH}_4$  fluxes mainly varied between -12 and  $-94 \mu\text{g C m}^{-2} \text{ h}^{-1}$  (Castaldi and Fierro, 2005).

Passive night-time warming increased  $\text{CH}_4$  uptake by  $10 \mu\text{g C m}^{-2} \text{ h}^{-1}$  on average, corresponding to a rise in the uptake rate of about 20% (Fig. 4a). Enhanced  $\text{CH}_4$  uptake in response to warming was also observed in studies conducted in a mixed deciduous forest and a spruce-fir forest (Peterjohn et al., 1994; Rustad and Fernandez, 1998). The warming treatment raised mean daytime soil temperature by  $0.39^\circ\text{C}$  (Table 1). In addition, the warming treatment reduced soil moisture in the 0-20 cm soil layer by 0.99 vol%, thus potentially warming could

have affected the CH<sub>4</sub> flux solely via changes in soil moisture. However, most likely the CH<sub>4</sub> uptake was stimulated by changes in both variables. Our analysis revealed that a 0.39 °C-increase in soil temperature and a 0.99 vol%-reduction of soil moisture would increase the CH<sub>4</sub> uptake to almost the same extent, *i.e.* by 0.7 and 0.9 µg CH<sub>4</sub>-C m<sup>-2</sup> h<sup>-1</sup>, respectively. However, together the changes in these two variables only explained 16% of the observed rise in CH<sub>4</sub> uptake in response to warming. Thus, other parameters than soil temperature and soil moisture were apparently also involved in stimulating the net CH<sub>4</sub> uptake in the warmed plots.

In the full-factorial study, soil temperature appeared more important than soil moisture in controlling the CH<sub>4</sub> oxidation in the sandy soil at our heathland site. This is in contrast to other studies in temperate ecosystems on aerated soils, where soil moisture had a stronger influence on the CH<sub>4</sub> uptake compared to soil temperature (McHale et al., 1998; Peterjohn et al., 1994; Rustad and Fernandez, 1998). In most ecosystems, CH<sub>4</sub> uptake is controlled by the physical diffusion of CH<sub>4</sub> from the atmosphere to the soil layer, where CH<sub>4</sub> oxidation takes place, and soil moisture is a variable that influences the diffusive transport of CH<sub>4</sub> (Borken et al., 2006; King, 1997). On the contrary, CH<sub>4</sub> uptake seems to be less controlled by biotic factors regulated by for instance soil temperature as uptake rates are relatively similar across many different ecosystems worldwide (Billings et al., 2000; King, 1997). In our study, a likely reason for the weaker negative influence of soil moisture on CH<sub>4</sub> fluxes compared to the positive influence of soil temperature could be that the sandy soil at the site is very well-drained. At field capacity, about 60 % of the pore space is air-filled in the 0-15 cm layer of the mineral soil, thus CH<sub>4</sub> diffusion is seldom limited by water-filled soil pores.

Accordingly, the drought treatment had no effect on the CH<sub>4</sub> flux although this treatment reduced the soil water content in the 0-20 cm layer during three out of nine measuring campaigns. The period of experimental drought in May-June 2007 also showed that the drought treatment

had limited direct or indirect effects on the CH<sub>4</sub> flux as the uptake was only affected by drought during one out of three occasions, and since no delayed drought response in the CH<sub>4</sub> flux occurred from June to October 2007. In fact, the methanotrophic activity was reduced due to drought stress on the first measuring day in the drought period (30 May; Fig. 7c). During the last part of the experimental drought, the methanotrophs were also drought stressed in the non-drought treated plots, as the CH<sub>4</sub> uptake increased across all treatment on the first day after rewetting (26 June). A similar response was observed by Priemé and Christensen (1999) at onset of the rainy season in the African savanna.

In summary, the methanotrophs were more susceptible to dry conditions in May-June 2007 compared to the drought period in July-August 2006, where the highest CH<sub>4</sub> uptake rates were recorded on 17-18 July at soil moisture levels of about 5 vol% in the 0-20 cm layer (Fig. 3a). One possible reason for the difference could be the relatively dry spring of 2007, where the site only received 39 mm of rainfall during the last two months prior to the experimental drought (Fig. 2a), whereas the pre-drought precipitation in 2006 was 122 mm. Thus, presumably the methanotrophs were only able to cope with dry conditions for a limited period.

The complex control of soil moisture on CH<sub>4</sub> fluxes was illustrated by the reduction in CH<sub>4</sub> consumption rates as the soil water content continued to rise during rewetting in 2007, resulting in net CH<sub>4</sub> emissions in some plots on the second day after rewetting (27 June; Fig. 7c). The CH<sub>4</sub> flux therefore seemed to be controlled by soil moisture in a non-linear way in the drought/rewetting study. A similar relationship between CH<sub>4</sub> uptake and soil moisture was found in a Dutch heather grassland on sandy soil (van den Pol-van Dasselaar et al., 1998). Furthermore, Dijkstra et al. (2010) eliminated constraints on CH<sub>4</sub> uptake due to gas diffusion and showed that methanotrophic activity increased under elevated CO<sub>2</sub>, partly due to an increase in soil moisture.

In January 2007, the CH<sub>4</sub> uptake was reduced in the plots exposed to elevated CO<sub>2</sub> compared to the ambient CO<sub>2</sub> plots. A root in-growth study conducted at the site showed that elevated atmospheric CO<sub>2</sub> stimulated root growth of both *Deschampsia* and *Calluna* (Marie F. Arndal, pers. comm.). Thus, a likely reason for the temporary CO<sub>2</sub> effect on CH<sub>4</sub> fluxes is increased methanogenesis in soils under elevated atmospheric CO<sub>2</sub>, fuelled by increased winter root decay resulting from a larger root biomass. McLain and Ahmann (2008) also suggested that reduced net CH<sub>4</sub> uptake under elevated CO<sub>2</sub> was due to increased CH<sub>4</sub> production. Another possible reason is reduced CH<sub>4</sub> oxidation in the CO<sub>2</sub> plots caused by increased soil water content in the upper most soil horizon (0-6 cm), which was observed in March 2007. Similar mechanisms were also put forward in other studies (Ambus and Robertson, 1999; Baggs and Blum, 2004).

Summarizing treatment effects on CH<sub>4</sub> fluxes, prolonged summer drought did not increase the CH<sub>4</sub> uptake as hypothesized because soil moisture at the site was already low and therefore not an obstacle for CH<sub>4</sub> diffusion. The warming treatment did increase the CH<sub>4</sub> uptake as hypothesized, however apparently not only via the effect on soil moisture that we expected, but also through a direct stimulation of the methanotrophic activity by increased soil temperatures and due to other unknown factors. Finally, elevated atmospheric CO<sub>2</sub> did decrease the CH<sub>4</sub> uptake as hypothesized, but only in January 2007 and the exact mechanism is not well understood. In the three-factor combination TDCO<sub>2</sub>, the positive effect of warming on the CH<sub>4</sub> uptake was partly outweighed by the reduced CH<sub>4</sub> uptake under elevated CO<sub>2</sub> during winter, resulting in a slight but insignificant increase in the CH<sub>4</sub> uptake compared to the ambient treatment. We therefore accept the fourth hypothesis that individual treatment effects counteract each other if in opposite directions.

#### 4.2 $N_2O$ response to elevated $CO_2$ combined with drought or warming

Heterotrophic nitrification may be a significant source of  $N_2O$  in soils with low pH and high availability of oxygen and organic material (Wrage et al., 2001). This process possibly contributed to the  $N_2O$  emission from the acidic and well-aerated sandy soil at our site, along with autotrophic nitrification and denitrification.

Neither elevated  $CO_2$ , drought or warming affected the  $N_2O$  flux as main treatments when analyzed across all measuring campaigns in the full-factorial study, but the  $N_2O$  flux responded to combinations of these treatments (Fig. 4b). Thus, in this study the effect of treatment combinations could not be predicted from the effects of the single treatments. The  $N_2O$  emission ceased when elevated  $CO_2$  was combined with drought, whereas increased  $N_2O$  emissions occurred when elevated  $CO_2$  and warming was combined. Of the three main treatments, warming affected soil moisture most frequently; however the reduction of soil moisture in the warmed plots could not explain the increase of  $N_2O$  emissions in the  $TCO_2$  treatment. Furthermore, the poor indications of  $CO_2$  effects on soil moisture via improved plant water use efficiency as well as the temporally limited drought effects on soil water suggested that the  $N_2O$  responses in the  $DCO_2$  and  $TCO_2$  treatments were not related to differences in soil water content between treatments, but to one or more unknown parameters that will be discussed below.

Potential nitrification and denitrification rates were increased in the warmed plots (Fig. 6), which indicates an increased content in the soil of enzymes involved in the two processes. This is in contrast to studies in California and Wales, where natural and semi-natural grasslands were exposed to elevated temperatures for about four years with no effect on nitrifying or denitrifying enzyme activities (Barnard et al., 2004, 2006). In line with our results on potential nitrification and denitrification, warming increased the concentration of  $NO_3^-$  in soil water, counteracting negative effects of drought and  $CO_2$  (Fig. 5). Additionally, compiled data from our site showed



that microbial  $\text{NH}_4^+$  consumption was increased by warming (Larsen et al., 2011). In contrast, drought reduced gross N mineralization and the fauna-related part of N mineralization, which included N excretion by soil fauna and turnover of the soil fauna biomass. Thus, in general N turnover was enhanced in the warmed plots, whereas drought tended to reduce N turnover at the site. Apparently, the effects of drought and warming on N turnover only affected the  $\text{N}_2\text{O}$  flux in the full-factorial study when each of the two treatments was combined with elevated  $\text{CO}_2$ . Elevated  $\text{CO}_2$  therefore seemed to trigger the  $\text{N}_2\text{O}$  response to drought and warming.

Elevated  $\text{CO}_2$  is known to stimulate rhizodeposition (Allard et al., 2006; Pendall et al., 2004). Studies at our heathland site showed increased root growth (Marie F. Arndal, pers. comm.) and increased belowground respiration in response to elevated  $\text{CO}_2$  (Merete B. Selsted, pers. comm.), indicating enhanced carbon allocation belowground and possibly also increased rhizodeposition. In the  $\text{TCO}_2$  treatment, a larger input of labile C compounds possibly stimulated the denitrifying microorganisms in combination with the increased N availability caused by the warming treatment, resulting in enhanced  $\text{N}_2\text{O}$  production by denitrification (Fig. 4b). Increased denitrification in the  $\text{TCO}_2$  treatment, however, was not supported by measurements of potential denitrification (Fig. 6b). An alternative source of  $\text{N}_2\text{O}$  in the  $\text{TCO}_2$  treatment could be heterotrophic nitrification that would be stimulated by the same mechanism and may have a higher  $\text{N}_2\text{O}$  product ratio than autotrophic nitrification (Papen et al., 1989). In contrast, increased C input via rhizodeposition probably shifted the balance even further between supply of reductant (*e.g.* organic carbon) and oxidant (*e.g.*  $\text{NO}_3^-$ ) for denitrifiers in the  $\text{DCO}_2$  treatment, where the N availability was already low due to the negative effects of drought on N turnover. In denitrification, nearly all nitrogen oxide is reduced to  $\text{N}_2$  when the availability of reductant exceeds the supply of oxidant to a great extent (Hutchinson and Davidson, 1993). This could be the reason for the low  $\text{N}_2\text{O}$  emission in the  $\text{DCO}_2$  treatment (Fig. 4b), despite the potential

activity of enzymes involved in nitrification and denitrification being unaffected (Fig. 6). In line with our results, Baggs et al. (2003) found that elevated atmospheric CO<sub>2</sub> caused increased N<sub>2</sub>O emission in highly N fertilized swards, whereas elevated CO<sub>2</sub> tended to reduce the N<sub>2</sub>O emission in low N swards. Furthermore, in nitrogen poor meadow mesocosms, Kanerva et al. (2007) studied the effect of elevated CO<sub>2</sub> via increased C input to the soil, while controlling soil moisture. They concluded that, in low N soils, the greater C availability under elevated CO<sub>2</sub> does not lead to greater N<sub>2</sub>O emissions.

In our study, all three treatments were combined in order to simulate the climatic and atmospheric conditions in Denmark in year 2075. The positive effect of TCO<sub>2</sub> and the negative effect of DCO<sub>2</sub> on the N<sub>2</sub>O flux counteracted each other in the TDCO<sub>2</sub> treatment, resulting in no change in the N<sub>2</sub>O flux compared to the current climatic and atmospheric conditions given by the ambient treatment, A. The lack of major changes in the N<sub>2</sub>O emission from the TDCO<sub>2</sub> treatment is in line with a study on extensively managed grassland monoliths, which were exposed to elevated temperature, summer drought and elevated atmospheric CO<sub>2</sub> using an additive experimental design (Cantarel et al., 2011).

The strong control of soil moisture on the temporal changes in N<sub>2</sub>O fluxes appeared from both the full-factorial study and the drought/rewetting study. The one-day delay in the response of N<sub>2</sub>O fluxes to rewetting observed in the drought/rewetting study was probably related to threshold levels for N<sub>2</sub>O production, meaning that the soil water content needed to reach a certain level before the development of anaerobic zones enabled the denitrification to occur (Smith et al., 1998) (Fig. 7b,d). In addition to slowing the gas diffusion, rewetting of soil may promote the development of anaerobic zones by stimulating the respiratory activity, leading to enhanced O<sub>2</sub> consumption (Ruser et al., 2006). The source of the short-term N<sub>2</sub>O peak was possibly NO<sub>3</sub><sup>-</sup> that accumulated in the soil during the preceding drought period.

Revisiting our hypotheses, we expected that prolonged summer drought as main treatment would reduce the N<sub>2</sub>O emission, which we did not find when analysing across all measuring campaigns in the full-factorial study. However, temporary drought effects occurred during the experimental drought periods in 2006 (full-factorial study) and 2007 (drought/rewetting study). Furthermore, in combination with elevated CO<sub>2</sub>, drought reduced the N<sub>2</sub>O emission. In line with our hypotheses, we found no effect on the overall N<sub>2</sub>O flux of elevated CO<sub>2</sub> or warming, but in combination the two treatments enhanced the N<sub>2</sub>O emission. Although the outcome of the two-factor combinations, DCO<sub>2</sub> and TCO<sub>2</sub>, could not be predicted from the single treatments, the response in the three-factor combination fitted well with our last hypothesis that two opposing two-factor responses would counteract each other.

#### *4.3 Depth distribution of microbial activity*

In contrast to field measurements conducted in October 2007, where significant N<sub>2</sub>O uptake occurred in some plots, laboratory incubations of soil from the 0-8 cm soil layer sampled in November 2007 showed negligible N<sub>2</sub>O reductase activity. One explanation could be that the N<sub>2</sub>O reduction took place in soil layers below the 0-8 cm layer. In a spruce forest, Goldberg and Gebauer (2009) also found microbial N<sub>2</sub>O consumption in the mineral horizon.

In dry soils, methane oxidation may occur well below the soil surface (Castaldi and Fierro, 2005 and references within). However, in the drought/rewetting study drought stress on the methanotrophs was released on the first day after rewetting (26 June; Fig. 7c) following a rather modest rainfall of 6 mm that caused a general rise in soil moisture in the 0-20 cm soil layer, but not in the 20-60 cm layer in all plots. This suggests that the main zone of CH<sub>4</sub> oxidation was localised in the upper part of the soil profile, which was supported by a stronger influence on the CH<sub>4</sub> flux of soil moisture in the 0-20 cm layer compared to moisture in the 20-60

cm layer. Studies in forest soils also showed that CH<sub>4</sub> oxidation primarily occurred within the upper 10 cm of the soil profile (Bender and Conrad, 1994; King, 1997).

On 27 June and 2 July 2007, high rates of CH<sub>4</sub> emissions (above 100 µg C m<sup>-2</sup> h<sup>-1</sup>) occurred in six specific plots across treatments. These CH<sub>4</sub> hotspots were presumably triggered by the high soil water content reducing CH<sub>4</sub> oxidation and producing anaerobic microsites combined with high local availability of substrates for methanogenesis. On 2 July, the plot with the highest CH<sub>4</sub> emission also had the highest N<sub>2</sub>O emission and soil water content in 0-6 cm, supporting anaerobic conditions as an important driver for the CH<sub>4</sub> hotspots. The abundance of *Deschampsia* and *Calluna* varied between plots at our heathland site, but the vegetation composition had no effect on the measured CH<sub>4</sub> and N<sub>2</sub>O fluxes in any of the studies.

#### 4.4 Conclusions

Our study showed that warming as main factor increased the CH<sub>4</sub> uptake by about 20 %, presumably due to the enhancing effects of increased soil temperatures and reduced soil moisture on the microbial CH<sub>4</sub> oxidation process as well as some unknown factors. Elevated concentrations of atmospheric CO<sub>2</sub> had no overall effect on the CH<sub>4</sub> flux, but reduced the CH<sub>4</sub> uptake during one measuring campaign in the winter season. In combination, the stimulating effect of warming and the episodic reducing effect of CO<sub>2</sub> on the CH<sub>4</sub> uptake resulted in a modest, but insignificant, increase in the CH<sub>4</sub> uptake when comparing the multifactor treatment including elevated CO<sub>2</sub>, warming and summer drought with the ambient treatment. Depending on the duration of winter decline in CH<sub>4</sub> uptake under elevated CO<sub>2</sub>, CH<sub>4</sub> oxidation in temperate ecosystems on well-aerated soil could potentially have a negative feedback on global climate change in future.

The study indicated that the N<sub>2</sub>O flux in nitrogen poor natural ecosystems on well-aerated soils will probably not change under future climatic and atmospheric conditions. This apparent lack of response is the product of the complex interaction between the climate change parameters affecting the conditions for N<sub>2</sub>O production in opposite directions. Overall, this study highlights the importance of evaluating climate change parameters in multifactor treatments as the response of CH<sub>4</sub> and N<sub>2</sub>O flux rates to different two- and three-factor combinations may not be predicted from the responses to the individual treatments, and furthermore the effects of individual treatments may negate each other if they act in opposite directions.

The greenhouse gas fluxes reported here cover short-term (1-2 year) responses to climate change. We anticipate that after longer experimental manipulation, the treatment effects could differ from those initially observed. For example, the increased carbon input via stimulation of photosynthesis under elevated CO<sub>2</sub> (Albert et al., 2011a, 2011b) may result in changes in the quantity or character of the ecosystem carbon pools. Furthermore, the increased N turnover observed in response to warming (Larsen et al., 2011), also a short-term response, may be influenced by the properties of the pre-experimental organic matter. Therefore, a new equilibrium N turnover rate may develop as the properties of the soil organic matter also equilibrate; a process controlled by the continuous supply and decomposition of organic matter. Both the change in carbon pools and the potential change in the N turnover rate would alter the predictions for the future fluxes of N<sub>2</sub>O and possibly CH<sub>4</sub>.

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Table 1. Absolute changes in soil temperature and soil moisture in response to the three main factors, elevated temperature (T), drought (D) and elevated atmospheric CO<sub>2</sub> concentrations (CO<sub>2</sub>), and significant treatment interactions during measuring campaigns in the full-factorial study

	Main factor			Interaction
	T	D	CO <sub>2</sub>	
Mean daytime soil temperature at 5 cm depth ( $\Delta^{\circ}\text{C}$ )	+ 0.39 ****	- 0.07 *	ns	CO <sub>2</sub> ×T ****
Soil moisture in 0-20 cm ( $\Delta$ vol%)	- 0.99 ****	- 0.85 ***	ns	CO <sub>2</sub> ×D **, CO <sub>2</sub> ×D×T *
Soil moisture in 20-60 cm ( $\Delta$ vol%)	- 0.84 **	- 0.79 **	ns	

Significance levels are: \*\*\*\* for  $P \leq 0.0001$ ; \*\*\* for  $P \leq 0.001$ ; \*\* for  $P \leq 0.01$ ; \* for  $P \leq 0.05$ ; ns for not significant.

Table 2. Parameter estimates for a repeated measures model used to investigate the influence on CH<sub>4</sub> fluxes (µg C m<sup>-2</sup> h<sup>-1</sup>) of soil temperature at 5 cm depth (°C) and soil moisture in the 0-20 cm layer (vol%)

Parameter	Estimate	SE	P-value
μ	12.25	0.48	<0.0001
α(T = 0)	-0.35	0.20	0.082
α(T = 1)	0	.	.
β(D = 0)	0.28	0.20	0.17
β(D = 1)	0	.	.
γ(CO <sub>2</sub> = 0)	0.34	0.26	0.25
γ(CO <sub>2</sub> = 1)	0	.	.
δ(Temp)	0.070	0.019	0.0004
ε(Moist)	-0.034	0.019	0.074

Model structure:

$$(-CH_4+110)^{0.5} = \mu + \alpha(T) + \beta(D) + \gamma(CO_2) + \delta \cdot Temp + \varepsilon \cdot Moist$$

The main factors were elevated temperature (T), drought (D) and elevated atmospheric CO<sub>2</sub> concentrations (CO<sub>2</sub>). All two- and three-factor interactions had P-values >0.25 and were removed from the model.

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### Figure legends

Fig. 1 The arrangement of the field experiment at Brandbjerg, Denmark (a) and the layout of a single block of the experiment (b). In part (a), the octagons are labelled 1 through 12, with consecutive pairs belonging to the same experimental block. Octagons 2, 4, 5, 8, 10 and 12 received elevated CO<sub>2</sub> concentrations. The boardwalks are shown by solid lines and the locations of the two meteorological stations are shown as M1 and M2. The two rectangles represent buildings that house computers, control systems and field laboratories. In part (b), the extension direction of the curtains that provide the night-time warming (T) and drought (D) treatments is shown on the upper octagon. One octagon is at ambient concentrations of CO<sub>2</sub> while the other received elevated concentrations of CO<sub>2</sub> from the Free Air Carbon Enrichment (FACE) system.

Fig. 2 Temporal variation in rainfall (a), soil moisture (b) and soil temperature (c) during measuring campaigns in the full-factorial study. Soil moisture in the 0-20 cm and 20-60 cm soil layers as well as mean daytime soil temperature at 5 cm depth were measured in the ambient treatment (n=6; means ± SE). Periods of experimental drought are indicated by shaded areas.

Fig. 3 Temporal variation in CH<sub>4</sub> (a) and N<sub>2</sub>O fluxes (b) during measuring campaigns in the full-factorial study. Fluxes of CH<sub>4</sub> are presented for warmed and unwarmed treatments separately (n=24; means ± SE). Fluxes of N<sub>2</sub>O in three treatments are presented, viz. the ambient treatment (A), the combination of drought and elevated CO<sub>2</sub> (DCO<sub>2</sub>) and the combination of warming and elevated CO<sub>2</sub> (TCO<sub>2</sub>) (n=6; means ± SE). The remaining five treatments were left out for clarity. Periods of experimental drought are indicated by shaded areas.



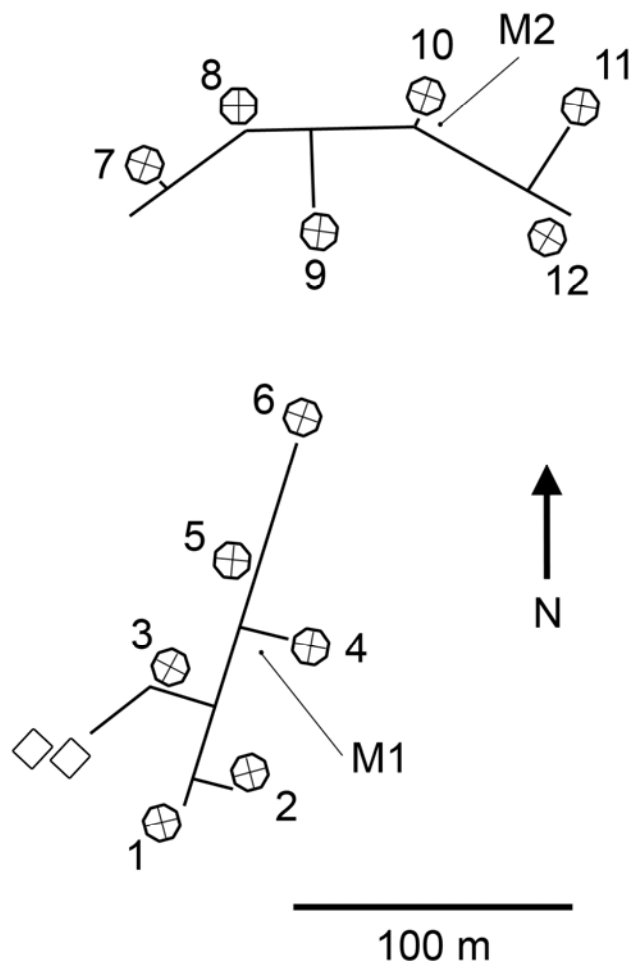
Fig. 4 Mean CH<sub>4</sub> fluxes (a) and mean N<sub>2</sub>O fluxes (b) across measuring campaigns in the full-factorial study for the ambient treatment (A) in addition to elevated CO<sub>2</sub> (CO<sub>2</sub>), drought (D) and elevated temperature (T) as single treatments and in all combinations (CH<sub>4</sub>, n=54; N<sub>2</sub>O, n=66; means ± SE).

Fig. 5 Annual mean of NO<sub>3</sub><sup>-</sup> concentrations in soil water collected at 5 cm depth in 2007 (n=6; means ± SE)

Fig. 6 Potential nitrification (a) and potential denitrification (b) in soil collected in November 2007 in the ambient treatment (A) and in elevated CO<sub>2</sub> (CO<sub>2</sub>), drought (D) and elevated temperature (T) as single treatments as well as in all combinations (n=6; means ± SE). In part (a), bars with same letter are not significantly different. The pair wise comparisons of the eight treatments are based on differences of least squares means, and are enabled by the significant CO<sub>2</sub>×D×T interaction.

Fig. 7 Temporal variation in rainfall (a), soil moisture in 0-6 cm (b), CH<sub>4</sub> fluxes (c) and N<sub>2</sub>O fluxes (d) during measuring campaigns in the drought/rewetting study measured in the treatments ambient (A), elevated CO<sub>2</sub> (CO<sub>2</sub>), drought (D), combined drought and elevated CO<sub>2</sub> (DCO<sub>2</sub>) and the combination of elevated temperature, drought and CO<sub>2</sub> (TDCO<sub>2</sub>) (n=6; means). Standard error bars are not indicated for clarity. The mean coefficient of variation (CV) across treatments and time for soil moisture, CH<sub>4</sub> and N<sub>2</sub>O fluxes were 0.3, 3.0 and 3.6, respectively. The drought period is indicated by shaded areas.

(a)



(b)

